

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C. 20231
 ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 04 November 1999 (04.11.99)	
International application No. PCT/IL99/00112	Applicant's or agent's file reference 116803.8 RS
International filing date (day/month/year) 24 February 1999 (24.02.99)	Priority date (day/month/year) 25 February 1998 (25.02.98)
Applicant KEINAN, Ehud et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

22 September 1999 (22.09.99)

☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer C. Carrié Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCTNOTIFICATION OF WITHDRAWAL
OF PRIORITY CLAIM(PCT Rule 90bis.3 and
Administrative Instructions, Section 415(a) and (b))

To:

REINHOLD COHN AND PARTNERS
P.O. Box 4060
61040 Tel Aviv
ISRAEL

Date of mailing (day/month/year) 14 August 2000 (14.08.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 116803.8 RS	
International application No. PCT/IL99/00112	International filing date (day/month/year) 24 February 1999 (24.02.99)
Applicant KEINAN, Ehud	

1. The applicant is hereby notified that the **priority claim made in the international application has been withdrawn** in accordance with a notice of withdrawal received from the applicant on:

03 August 2000 (03.08.00)

The attention of the applicant is drawn to the fact that the withdrawal of the priority claim will result in the re-calculation of time limits which have not already expired (see Rule 90bis.3(d)).

2. ☐ In the case where **multiple priorities** have been claimed, the above action relates to the following priority claim(s):

3. A copy of this notification has been sent to the receiving Office and to:

- ☐ the International Searching Authority (*where the international search report has not yet been issued*)
- ☒ the designated Offices (*which have already been notified of the receipt of the record copy*)
- ☐ the International Preliminary Examining Authority

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer H. Zhou
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

REINHOLD COHN AND PARTNERS
P.O. Box 4060
61040 Tel-Aviv
ISRAEL

RECEIVED

21-11-1999

REINHOLD COHN & PARTNERS

PCT

**NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)**

Date of mailing
(day/month/year) 16.11.1999

Applicant's or agent's file reference
116803.8 LK

IMPORTANT NOTIFICATION

International application No.
PCT/IL99/00112

International filing date (day/month/year)
24/02/1999

Priority date (day/month/year)
25/02/1998

Applicant
KEINAN, Ehud et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523650 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Danti, B

Tel. +49 89 2399-2879



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL99/00112

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

Description, pages:

1-9 as originally filed

Claims, No.:

1-32 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims 1-32
	No: Claims
Inventive step (IS)	Yes: Claims 1-32
	No: Claims
Industrial applicability (IA)	Yes: Claims 1-32
	No: Claims

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IL99/00112

2. Citations and explanations

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IL99/00112

1. The subject matter of the claims fulfills the requirements of both Articles 33(2) and 33(3) PCT. The methods and kits based upon explosive peroxide detection enzymatically is new in the light of the documents cited in the ISR. Furthermore the method is considered to involve an inventive step because using an enzyme catalysed colour reaction for the direct detection of explosive peroxides is not suggested in any of the documents cited. Neither would a combination of these documents lead the skilled person directly to the methods and kits claimed.

PATENT COOPERATION TREATY

PCT

18

REC'D 18 NOV 1999

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



Applicant's or agent's file reference 116803.8 LK	FOR FURTHER ACTION <small>See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)</small>	
International application No. PCT/IL99/00112	International filing date (day/month/year) 24/02/1999	Priority date (day/month/year) 25/02/1998
International Patent Classification (IPC) or national classification and IPC C12Q1/28		
Applicant KEINAN, Ehud et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

 These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:
 - I ☒ Basis of the report
 - II ☐ Priority
 - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application

Date of submission of the demand 22/09/1999	Date of completion of this report 16.11.1999
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hinchliffe, P Telephone No. +49 89 2399 8431 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL99/00112

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-9 as originally filed

Claims, No.:

1-32 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-32
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-32
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IL99/00112

2. Citations and explanations

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IL99/00112

1. The subject matter of the claims fulfills the requirements of both Articles 33(2) and 33(3) PCT. The methods and kits based upon explosive peroxide detection enzymatically is new in the light of the documents cited in the ISR. Furthermore the method is considered to involve an inventive step because using an enzyme catalysed colour reaction for the direct detection of explosive peroxides is not suggested in any of the documents cited. Neither would a combination of these documents lead the skilled person directly to the methods and kits claimed.

XP-002107241

4/7 - (C) FILE CAPLUS

STN CA Caesar accession number : 1380

AN - 1992:165556 CAPLUS

DN - 116:165556

TI - Enzyme-linked immunosorbent assay for the determination of
2,4,6-trinitrotoluene and related nitroaromatic compounds

IN - Keuchel, Claudia; Weil, Ludwig; Niessner, Reinhard

CS - Inst. Hydrochem., Tech. Univ. Munich, Munich, D-8000/70, Germany

SO - Anal. Sci. (1992), 8(1), 9-12
CODEN: ANSCEN; ISSN: 0910-6340

DT - Journal

LA - English

AB - A sensitive ELISA for the detn. of the explosive
2,4,6-trinitrotoluene (TNT) and other nitroarom. compds. was developed.
As tracer, a trinitrophenyl-deriv. conjugated to horseradish
peroxidase was used. With this competitive assay, performed in
polystyrene microtiter plates, TNT can be detected within the range of
0.02 .mu.g/L and 20 .mu.g/L. Cross-reactivities at the center points (5
values) of several nitroarom. compds. were detd. Addnl., the
cross-reactivities at different concns. of the analytes were stated to
yield a more realistic estn. of the interferences. Finally, the
applicability of the immunoassay to the detn. of TNT in water samples wa
assessed.

P.D. 1992.....	(1)
p. =	

XP-002107242

5/7 - (C) FILE CAPLUS
STN CA Caesar accession number : 1381
AN - 1992:10880 CAPLUS
DN - 116:10880
TI - Biological decontamination of water contaminated with explosives
by Phanerochaete chrysosporium
IN - Fernando, Tudor; Aust, Steven D.
CS - Biotechnol. Cent., Utah State Univ., Logan, UT, 84322-4700, USA
SO - Gas, Oil, Coal, Environ. Biotechnol. 3, [Pap. IGT's Int. Symp.], 3rd
(1991), Meeting Date 1990, 193-206. Editor(s): Akin, Cavit; Smith, Jare
Publisher: IGT, Chicago, Ill.
CODEN: 57IIA8
DT - Conference
LA - English
AB - Biodegrdn. of TNT, RDX, HMX was demonstrated in contaminated water by th
white rot fungus Phanerochaete chrysosporium. At an initial concn. of 3
ppm TNT and RDX, 46-51% and 67% of [14C]TNT and [14C]RDX, resp., were
converted to 14CO2 over a 30-day incubation period. The amt. of HMX
degraded by cultures contg. 1000 ppm was 20%, over a 30-day period. HPL
anal. of the culture exts. of [14C]TNT and HMX revealed polar metabolite
formation, but no metabolites were obsd. in [14C]RDX contaminated
cultures. In control cultures where the fungus was not included, 97-98%
of the initial radioactivity added to the cultures was recovered as
undegraded [14C]TNT or [14C]RDX and in the case of HMX as non-radiolabel
HMX. When [14C]TNT or [14C]RDX were incubated with a pure lignin
peroxidase (LiP) isoenzyme, i.e., LiP isoenzyme H2, metabolites
were formed that were more polar than TNT or RDX. Metabolites formed fr
the incubations of [14C]TNT with LiP H2 were identical to those formed i
cultures. One major metabolite was formed upon incubation of [14C] RDX
with LiP H2, but none were obsd. in culture exts. Enzymic incubation of
[14C]TNT with Mn dependent peroxidase (MnP) isoenzyme H4 did not
form 2 major metabolites that were obsd. with LiP H2 and in cultures.
However, identical metabolites were obsd. with enzymic incubations of RD
with LiP H2 and MnP H4.

PD. 1992	①
p. =	

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 116803.8 RS	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/IL 99/ 00112	International filing date (day/month/year) 24/02/1999	(Earliest) Priority Date (day/month/year) 25/02/1998
Applicant KEINAN, Ehud et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 99/00112

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 314 156 A (SECR DEFENCE) 17 December 1997 see the whole document	1, 16
A	WO 79 00122 A (FOSPUR LTD ;PITT M (GB); KEEN R (GB)) 22 March 1979 see page 19	1, 16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 June 1999

Date of mailing of the international search report

07/07/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Moreno, C

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US KEUCHEL, CLAUDIA ET AL: "Enzyme-linked immunosorbent assay for the determination of 2,4,6-trinitrotoluene and related nitroaromatic compounds" XP002107241 see abstract & ANAL. SCI. (1992), 8(1), 9-12 CODEN: ANSCEN;ISSN: 0910-6340, ---</p>	1,16
A	<p>DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US FERNANDO, TUDOR ET AL: "Biological decontamination of water contaminated with explosives by Phanerochaete chrysosporium" XP002107242 see abstract & GAS, OIL, COAL, ENVIRON. BIOTECHNOL. 3, 'PAP. IGT'S INT. SYMP.!', 3RD (1991), MEETING DATE 1990, 193-206. EDITOR(S): AKIN, CAVIT;SMITH, JARED PUBLISHER: IGT, CHICAGO, ILL. CODEN: 57IIA8, ---</p>	1,16
A	<p>GB 1 177 516 A (CHISSO CORPORATION) 14 January 1970 see the whole document -----</p>	1,16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IL 99/00112

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
GB 2314156	A	17-12-1997	CA 2257940 A	18-12-1997
			EP 0904531 A	31-03-1999
			WO 9747958 A	18-12-1997
			GB 2328740 A	03-03-1999

WO 7900122	A	22-03-1979	EP 0007348 A	06-02-1980

GB 1177516	A	14-01-1970	JP 52034607 B	05-09-1977
			BE 699368 A	16-11-1967
			DE 1618245 A	11-03-1971
			FR 1525596 A	06-09-1968
			NL 6707739 A	04-12-1967

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/28	A1	(11) International Publication Number: WO 99/43846 (43) International Publication Date: 2 September 1999 (02.09.99)
(21) International Application Number: PCT/IL99/00112 (22) International Filing Date: 24 February 1999 (24.02.99) (30) Priority Data: 123451 25 February 1998 (25.02.98) IL (71)(72) Applicants and Inventors: <u>KEINAN, Ehud</u> [IL/IL]; Moran Street 8, 10505 Timrat (IL). <u>ITZHAKY, Harel</u> [IL/IL]; P.O. Box 430, 30300 Atlit (IL). (74) Agent: REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel Aviv (IL).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHOD AND KIT FOR THE DETECTION OF EXPLOSIVES (57) Abstract The present invention provides a method of detecting a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which method comprises dissolving said sample in a suitable organic solvent, contacting the solution with an aqueous solution of a strong acid capable of decomposing said explosive to release hydrogen peroxide, and contacting the resulting mixture with a peroxidase enzyme. The invention also provides a kit for use in the method of the invention.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

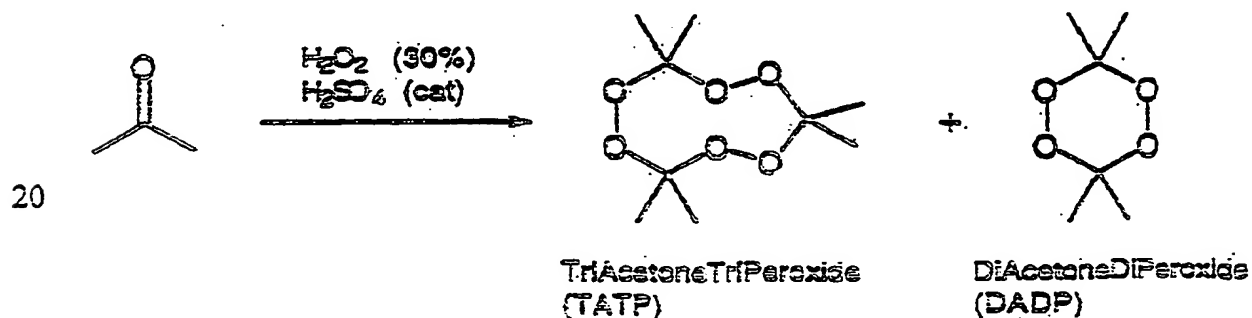
AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
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CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

METHOD AND KIT FOR THE DETECTION OF EXPLOSIVES

FIELD OF THE INVENTION

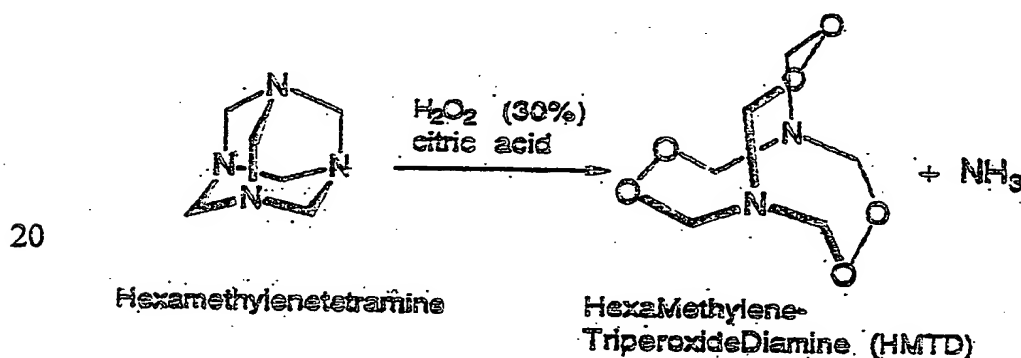
This invention concerns a method of detecting peroxide-based explosives and a kit for use in this method.

Improvised explosive devices based on peroxide containing materials have increasingly been used in recent years by various terrorist organizations, especially in Israel, as well as in the UK and the USA. The main reason is that such peroxide-based explosives can be easily "home-made" using inexpensive, readily available starting materials which can be purchased in most hardware and paint stores, even in bulk quantities. One class of such peroxide-based explosives can be easily produced by reacting various carbonyl compounds (e.g. ketones, aldehydes and their derivatives) with hydrogen peroxide under acid catalysis. For example, when a mixture of acetone, hydrogen peroxide and small amounts of a mineral acid, e.g. sulfuric acid, is left for several hours at room temperature, white crystals of triacetone triperoxide (hereinafter "TATP") and diacetone diperoxide (hereinafter "DADP") are formed by the following reaction :



These crystals are collected and can be washed with water or with 10% sodium carbonate solution. TATP and DADP are powerful initiators by themselves and can be used as the main filler in home-made detonators. They are quite unstable explosives and may explode under rough handling, scratching with metals or by sparks and open flame, even when they contain up to 25% water or even when immersed in water. The explosive intensity of TATP is approximately 5/8 that of TNT. This material is quite volatile, unless used shortly after its manufacture, should be stored in a cool, dark dry place. It has been reported that at room temperature TATP loses 2/3 of its weight within 14 days and at 50° C it evaporates completely within 40 days.

Another commonly used peroxide based explosive is hexamethylenetriperoxidediamine (hereinafter "HMTD"). It can be conveniently prepared by treating hexamethylenetetramine with hydrogen peroxide in the presence of a weak acid, such as citric acid, in order to neutralize the liberated ammonia. The reaction can be represented as follows:



HMTD is almost insoluble in water and in common organic solvents at room temperature. It is too active and too unstable to be of commercial use as an explosive.

Although many peroxide containing materials of the above-described type are known for more than 70 years, no satisfactory method for their detection has been suggested to date. The detection of peroxide-based explosives is particularly difficult because all these materials do not contain

nitro groups or any other nitrogen oxide functional groups. Since most of the currently available explosive detectors are based on the detection of nitro groups, they cannot be employed for detection of peroxide-based materials. Consequently, and in view of the increased use of such peroxide-based explosives by terrorists, especially in the Middle East as well as in other parts of the world, there exists an urgent need for highly sensitive methods and devices for the early detection of peroxide-based explosives and improvised explosive devices employing them.

10 OBJECT OF THE INVENTION

It is thus the object of the present invention to provide a reliable method for the fast and easy detection of peroxide-based explosives.

It is a further object of the invention to provide a portable kit for the simple yet reliable and selective detection and identification of peroxide based explosives.

SUMMARY OF THE INVENTION

The above object was achieved by the present invention which provides a method of detecting a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which method comprises dissolving said sample in a suitable organic solvent, contacting the solution with an aqueous solution of a strong acid capable of decomposing said explosive to release hydrogen peroxide, and contacting the resulting mixture with a peroxidase enzyme, a buffer to adjust the pH to such permitting action of the peroxidase enzyme and a substrate capable of being oxidized by the oxidant under the catalysis of the peroxidase enzyme to produce a pronounced change in a measurable physical parameter of the substrate.

The invention also provides, in a second aspect thereof, a kit for use in the method of the invention, comprising packaged organic solvent,

packaged aqueous solution of a strong acid, packaged buffer, packaged peroxidase enzyme and packaged substrate.

DETAILED DESCRIPTION OF THE INVENTION

5 In accordance with the method of the present invention, a sample to be tested for the presence of peroxide-based explosive, is dissolved in a suitable organic solvent and contacted with a strong acid in the presence of water, whereby any peroxide-containing material is decomposed to release free hydrogen peroxide. The resulting mixture is neutralized with a suitable buffer
10 and the presence of peroxide in the mixture is detected by means of a peroxidase enzyme and a substrate which is oxidized with a resulting pronounced change in a measurable physical parameter thereof. When a such a change is observed the result of the test is a positive indication of the presence of peroxide-based explosive in the tested sample.

15 The peroxide enzyme serves as a catalyst in the above process, so that only a small amount of the enzyme is needed for the oxidation of comparatively large amounts of the substrate. Any readily available peroxidase enzyme can be used, for example Horseradish peroxidase or soy bean peroxidase. A preferred peroxidase enzyme is the Horseradish peroxidase
20 which is highly selective for hydrogen peroxide.

 The invention also contemplates the use of a peroxidase enzyme which is immobilized on a solid support, for example on a probe which can be introduced into the test mixture or on the inner surface of a small receptacle wherein the test mixture can be introduced.

25 The term "substrate" is used, within the context of the present invention, to refer to a compound capable of being oxidized by hydrogen peroxide in the presence of a peroxidase enzyme to yield a product exhibiting a pronounced change in at least one measurable physical parameter as compared to the unoxidized compound. In accordance with a preferred embodiment of

the invention this measurable physical parameter is the colour or colour intensity.

Examples of organic solvents suitable for use in the method of the present invention are, for example, tetrahydrofuran, 1,4-dioxane, lower
5 alkanols, dimethylsulfoxide, N,N-dimethylformamide, carboxylic acids and sulfonic acids, especially acetic acid and trifluoroacetic acid. Preferred solvents are tetrahydrofuran, 1,4-dioxane and acetic acid.

Strong acids which can be used in the method of the present invention are, for example H_2SO_4 , HCl , HBr , HClO_4 , H_3PO_2 , H_3PO_3 , H_3PO_4
10 and HNO_3 . A preferred acid is sulfuric acid which can be used in concentrations of from about 5% to 95%, preferably from about 10% to about 50% by volume in water.

In accordance with an embodiment of the invention the sample to be tested can be introduced into a mixture of the organic solvent and the
15 aqueous solution of the strong acid.

In order to enable the enzymatic reaction of the peroxidase enzyme with the hydrogen peroxide and the substrate, it is necessary to neutralize the acidic mixture of the organic solvent and the aqueous strong acid containing the sample to be tested, so as to adjust its pH to a value between
20 about 5.0 to about 9.0. This can be achieved by a suitable buffer system which should be added to the mixture before the addition of the peroxidase enzyme or simultaneously therewith. In accordance with one embodiment of the invention the buffer is added to the mixture together with the substrate before the mixture is contacted with the peroxidase enzyme. Yet another preferred procedure
25 comprises adding the buffer, the substrate and the peroxidase enzyme simultaneously.

As suitable buffer systems for use in the method of the invention there may be mentioned citrate/phosphate buffer, acetate buffer, phthalate buffer, citrate buffer, phosphate buffer, imidazole buffer, triethanolamine

buffer, tris(hydroxymethyl)aminomethane buffer, bis-tris buffer and bis-tris propane buffer. A preferred buffer system is citrate/phosphate buffer 0.1M at pH 5.0.

Thus, in accordance with a preferred embodiment the invention provides a method of detecting a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which method comprises introducing said sample into a mixture of an organic solvent and an aqueous solution of a strong acid capable of decomposing said explosive to release hydrogen peroxide, and contacting the resulting mixture with a solution comprising a peroxidase enzyme, a buffer to adjust the pH to such permitting action of the peroxidase enzyme and a substrate capable of being oxidized by oxidant under the catalysis of the peroxidase enzyme to produce a pronounced change in the colour of the substrate or its colour intensity.

In this embodiment, the organic solvent is preferably acetic acid and the strong acid is preferably aqueous sulfuric acid 50% by volume. The preferred peroxidase enzyme is Horseradish peroxidase.

In accordance with a further aspect thereof, the invention provides a kit for the detection of a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which kit comprises packaged organic solvent, packaged aqueous solution of a strong acid, packaged buffer solution, packaged peroxidase enzyme and packaged substrate.

In a preferred kit according to the invention a mixture of the organic solvent and the aqueous solution of the strong acid are packaged together in the same container. In accordance with another modification of the kit according to the invention the buffer and the substrate are packaged together in the same container.

In accordance with a more preferred embodiment of the kit according to the invention the buffer, the peroxidase enzyme and the substrate are all packaged together. Conveniently the kit according to the invention

comprises a plurality of sealed ampoules each containing the peroxidase enzyme, optionally in admixture with the buffer and the substrate, in an amount sufficient for carrying out one test.

The kit according to the present invention may also include a plurality of small reaction vessels, for example small dishes or open receptacles, for carrying out the test therein.

The invention will now be described in more detail in the following non-limit examples.

10 Example 1

A few crystals of TATP (about 1-2mg) were placed in a shallow well, 0.1 ml of 1,4-dioxane 0.1ml was added and than 0.1ml of sulfuric acid 50% v/v in water. The reaction mixture was allowed to stand for 30 seconds before the substrate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt in 0.2ml of citrate/ phosphate buffer (0.1M at pH 5.0) was added, followed by 0.05ml of Horseradish peroxidase 5 mg/ml in citrate/phosphate buffer (0.1M at pH 5.0 ml.). An intense bluish-green colour developed in less than 30 seconds.

20

Example 2

The procedure of Example 1 was repeated using 1,4-dioxane as solvent instead of the tetrahydrofuran. The same result was obtained.

25

Example 3

The procedure of Example 1 was repeated using 2,7-diaminofluorene as the substrate. A blue-green colour developed in less than 30 seconds.

Example 4

The procedure of Example 1 was repeated using 3,3',5,5'-tetramethylbenzidine dihydrochloride as the substrate. An immediate blue colour formation was observed.

Example 5

The procedure of Example 1 was repeated using 5-aminosalicylic acid as the substrate. A brown colour developed rapidly.

Example 6

The procedure of Example 1 was repeated using o-phenylenediamine dihydrochloride as the substrate. An orange colour developed in less than 30 seconds.

Example 7

A few crystals of TATP (about 1-2mg) were placed in a shallow well, 0.1ml of a 1:1 mixture of sulfuric acid 50% v/v in water and acetic acid was added and the reaction mixture was allowed to stand for 30 seconds before 0.2ml of a 4:1 mixture of the substrate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt and the enzyme Horseradish peroxidase 5 mg/ml in citrate/phosphate buffer (0.1 M at pH 5.0) was added. An intense bluish-green colour developed in less than 30 seconds.

Example 8

The procedure of Example (7) was repeated using the substrates of Examples 3-6. The same results as in these examples were observed.

CLAIMS:

1. A method of detecting a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which method comprises
5 dissolving said sample in a suitable organic solvent, contacting the solution with an aqueous solution of a strong acid capable of decomposing said explosive to release hydrogen peroxide, and contacting the resulting mixture with a peroxidase enzyme, a buffer to adjust the pH to such permitting action of the peroxidase enzyme and a substrate capable of being oxidized by the oxidant
10 under the catalysis of the peroxidase enzyme to produce a pronounced change in a measurable physical parameter of the substrate.
2. A method according to Claim 1, wherein said physical parameter of the substrate is its colour or colour intensity
15
3. A method according to Claim 1 or 2, wherein the solvent is selected from tetrahydrofuran, 1,4-dioxane, lower alkanols, dimethylsulfoxide, N,N-dimethylformamide, carboxylic acids, especially acetic acid and trifluoroacetic acid, and sulfonic acids.
20
4. A method according to Claim 3, wherein the organic solvent is acetic acid.
5. A method according to any one of Claims 1 to 4, wherein the strong acid
25 is selected from H_2SO_4 , HCl , HBr , HClO_4 , H_3PO_2 , H_3PO_3 , H_3PO_4 and HNO_3 .
6. A method according to Claim 5, wherein the strong acid is H_2SO_4 .

7. A method according to Claim 6, wherein the concentration of the aqueous H_2SO_4 solution is from about 5% to 95%, preferably from about 10% to about 50% by volume.
- 5 8. A method according to any one of Claims 1 to 7, wherein the pH is adjusted by said buffer to about 5.0 to about 9.0.
9. A method according to any one of Claims 1 to 8, wherein the buffer is about 0.01 to 0.5 M citrate/phosphate buffer.
- 10 10. A method according to any one of Claims 1 to 9, wherein the peroxidase enzyme is Horseradish peroxidase.
11. A method according to any one of Claims 2 to 10, wherein the substrate
15 is selected from 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt, 2,7-diaminofluorene, 3,3',5,5'-tetramethylbenzidine and its dihydrochloride salt, 5-aminosalicylic acid, o-phenylenediamine and its dihydrochloride salt, 5-amino-2,3-dihydro-1,4-phthalazinedione, 3-amino-9-ethylcarbazole, 4-chloro-1-naphthol, 3,3'-diaminobenzidine, o-dianisidine and
20 its dihydrochloride salt, guaiacol and pyrogallol.
12. A method according to any one of Claims 1 to 11, wherein the sample is introduced into a mixture of the organic solvent and the aqueous solution of the strong acid.
- 25 13. A method according to any one of Claims 1 to 12, wherein the peroxidase enzyme is combined with the buffer prior to being contacted with said resulting mixture.

14. A method according to any one of Claims 1 to 12, wherein the buffer is combined with the substrate prior to being contacted with said resulting mixture.
- 5 15. A method according to any one of Claims 1 to 12, wherein said resulting mixture is contacted with a combination of the buffer, the peroxidase enzyme and the substrate.
- 10 16. A method of detecting a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which method comprises introducing said sample into a mixture of an organic solvent and an aqueous solution of a strong acid capable of decomposing said explosive to release hydrogen peroxide, and contacting the resulting mixture with a solution comprising a peroxidase enzyme, a buffer to adjust the pH to such permitting
15 action of the peroxidase enzyme and a substrate capable of being oxidized by the oxidant under the catalysis of the peroxidase enzyme to produce a pronounced change in the colour of the substrate or its colour intensity.
- 20 17. A method according to Claim 16, wherein the organic solvent is acetic acid.
18. A method according to Claims 16 or 17, wherein the strong acid is aqueous sulfuric acid.
- 25 19. A method according to any one of Claims 16 to 18, wherein the peroxidase enzyme is Horseradish peroxidase.

20. A kit for use in the method of Claim 1, comprising packaged organic solvent, packaged aqueous solution of a strong acid, packaged buffer, packaged peroxidase enzyme and packaged substrate.
- 5 21. A kit for use in the method of Claims 3 or 13, comprising a packaged mixture of organic solvent and aqueous solution of a strong acid, packaged buffer, packaged peroxidase enzyme and packaged substrate.
- 10 22. A kit according to Claims 20 or 21, wherein the buffer and the peroxidase enzyme are packaged together.
23. A kit according to Claims 20 or 21, wherein the buffer and the substrate are packaged together.
- 15 24. A kit according to Claims 20 or 21, wherein the buffer, the peroxidase enzyme and the substrate are packaged together.
- 20 25. A kit according to Claims 20 or 21, comprising a plurality of sealed ampoules each containing the peroxidase enzyme.
26. A kit according to Claim 22, comprising a plurality of sealed ampoules each containing the buffer and the peroxidase enzyme.
- 25 27. A kit according to Claim 24, comprising a plurality of sealed ampoules each containing the buffer, the peroxidase enzyme and the substrate.
28. A kit according to any one of Claims 20 to 27, wherein the organic solvent is acetic acid.

29. A kit according to any one of Claims 20 to 28, wherein the strong acid is aqueous sulfuric acid.
30. A kit according to any one of Claims 20-28, wherein the peroxidase
5 enzyme is Horseradish peroxidase.
31. A kit according to any one of Claims 20 to 30, wherein the buffer is citrate/phosphate buffer.
- 10 32. A kit according to any one of Claims 20 to 31, further comprising a plurality of small open receptacles for carrying out the test therein.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 99/00112

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 314 156 A (SECR DEFENCE) 17 December 1997 see the whole document	1,16
A	WO 79 00122 A (FOSPUR LTD ;PITT M (GB); KEEN R (GB)) 22 March 1979 see page 19	1,16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 99/00112

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US KEUCHEL, CLAUDIA ET AL: "Enzyme-linked immunosorbent assay for the determination of 2,4,6-trinitrotoluene and related nitroaromatic compounds" XP002107241 see abstract & ANAL. SCI. (1992), 8(1), 9-12 CODEN: ANSCEN;ISSN: 0910-6340,</p>	1,16
A	<p>DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US FERNANDO, TUDOR ET AL: "Biological decontamination of water contaminated with explosives by Phanerochaete chrysosporium" XP002107242 see abstract & GAS, OIL, COAL, ENVIRON. BIOTECHNOL. 3, 'PAP. IGT'S INT. SYMP.!, 3RD (1991), MEETING DATE 1990, 193-206. EDITOR(S): AKIN, CAVIT;SMITH, JARED PUBLISHER: IGT, CHICAGO, ILL. CODEN: 57IIA8,</p>	1,16
A	<p>GB 1 177 516 A (CHISSO CORPORATION) 14 January 1970 see the whole document</p>	1,16

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/IL 99/00112

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2314156 A	17-12-1997	CA 2257940 A	18-12-1997
		EP 0904531 A	31-03-1999
		WO 9747958 A	18-12-1997
		GB 2328740 A	03-03-1999
WO 7900122 A	22-03-1979	EP 0007348 A	06-02-1980
GB 1177516 A	14-01-1970	JP 52034607 B	05-09-1977
		BE 699368 A	16-11-1967
		DE 1618245 A	11-03-1971
		FR 1525596 A	06-09-1968
		NL 6707739 A	04-12-1967